METHODOLOGY FOR ELECTROPHORETIC ANALYSIS OF PHASEOLIN

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Phaseolin is the major seed storage protein of common bean (*Phaseolus vulgaris* L.). It is a globulin, soluble only at higher salt concentration, and which account for 35 to 50 % of total seed nitrogen (Ma & Bliss, 1978; Lioi, 1989). In genetic studies Brown *et al*. (1981a) observe that phaseolin is coded by a single complex loci (for 6 to 9 genes). This loci code for polypeptide subunits ranged from 43 kD to 54 kD, approximately (Brown *et al.*, 1981b). The alleles coding for the polypeptides of each phaseolin type were codominant and inherited like a single Mendelian unit.

Based on SDS/PAGE and 2D-IEF-SDS/PAGE, three electrophoretic types had been identified at the beginning among cultivated common bean, “S”, “T” and “C” types named after cultivars “Sanilac”, “Tendergreen” and “Contender”, respectively (Brown et al., 1981b). Two new banding patterns which had not been identified previously have been found among wild or cultivated forms, the “H” and “A” Phaseolin types after Peruvian landraces “Huevo de Huanchaco” and “Ayachucho”. The wild forms of the common bean show much greater diversity in phaseolin patterns than the cultivated forms (Gepts *et al.*, 1986). This heterogeneity may be attributed to higher levels of cross-pollination among wild beans (Vanderborght, 1982).

The study of biochemical markers as phaseolin permit enlarge the genetic maps (Gepts, 1988; Basset, 1991) and offer the opportunity to use it as genetic marker in the selection of material (Kelly and Miklas, 1999), in the studies of domestication, etc. Thus, phaseolin have been used as a marker to increase the protein content of common bean seeds (Kelly and Miklas, 1999). Electrophoretic analysis which detect the phenotypic molecular weight and isoelectric point changes resulting from genotypic divergence, are therefore a useful tool to analyse evolutionary relationships among phaseolin types and, by extension, among cultivars that produce them. Analysis of variation in electrophoretic patterns of seeds proteins is a useful method for establishing relationships among plant accessions within a species.

Electrophoretic analysis of seed proteins have also been used previously to identify or provide additional information on the wild progenitors of crop plants (Landizinsky & Hymowitz, 1979).

The electrophoretic variability of phaseolin of wild growing common beans from Middle America and the Andes was compared with that of landraces of the same regions using 1D-SDS-PAGE and 2D-IEF-SDS-PAGE. Two major findings emerged from the comparison of phaseolin electrophoretic variability (Table 7). Evans (1976) suggested two centres of domestication, one in Middle America giving rise to small-seeded cultivars and the other in the Andes leading to large-seeded cultivars. In Middle America wild forms showed both "S" type described earlier among cultivars (Brown *et al.*, 1981b) as well as "M" types (Table 7). Colombian wild common beans exhibited the novel "CH" and "B" types, whereas in the Southern Andes wild forms showed only the "T" type, described previously among common bean cultivars (Brown *et al.*, 1981b). Wild bean accessions from Middle America exhibited a high diversity of phaseolin electrophoretic patterns and showed phaseolin patterns not encountered among cultivated forms.

There was a correspondence in geographic distribution of phaseolin types between wild and cultivated common beans (Table 7). The cultivars with "S" and "T" phaseolin patterns predominated in Middle America and in the Southern Andes, respectively. The "B" phaseolin type was presented only in wild and cultivated common beans from Colombia. On the other hand, "C", "H" and "A" phaseolin types were found only among landraces of Andes. This correspondence can be attributed to various causes, multiple domestication, occasional outcrosses and escapes from cultivation. The multiple domestication was the primary cause for parallel geographical phaseolin variation between wild and cultivated common bean forms.

A relationship was observed between phaseolin type and seed type. Cultivars with "T", "C", "H", and "A" phaseolin patterns had larger seeds than cultivars with "S" and "B" phaseolin patterns (Gepts *et al.*, 1986; Gepts & Bliss, 1986). Combining phaseolin and seed size data, at least three independent domestications can be hypothesized. In Middle America, domestications gave rise to small-seeded, "S" phaseolin cultivars; in Colombia, to small-seeded, "B" phaseolin cultivars and in the Southern Andes, to large seeded "T" phaseolin cultivars. Because the low frequency of "B" phaseolin cultivars, Colombia might only be a minor or more recent domestication region (Gepts *et al.*, 1986). Colombia, located in the Northwestern part of South America, might possibly be a meeting place for the Middle American and Andean common bean germplasm as evidenced by the high frequencies of the "S" and "T" phaseolin types.

The origin of the "C", "H" and "A" phaseolin types remains to be determined. These three patterns were not found among Middle America cultivars and may therefore have originated in the Andes. The "C" type shared polypeptides with the

"S" and "T" types, suggesting that it might represent a rare recombinant between "T" and "S" types based on the intermediate nature of its polypeptide composition (Brown *et al.*, 1981b). Brown *et al.* (1981a) suggested that the "C" phaseolin genotype might have appeared through a translocation or an unequal crossing-over in a hybrid between two lines having "T" and "S" patterns. This event might have occurred after introduction of cultivars with "S" phaseolin types into Andean region.

Phaseolin patterns and seed type provide evidence for exchange of germplasm between Middle America and the Andes. The distribution of the different phaseolin types in the Andes, in general, and in Colombia, in particular, provides evidence for introduction of the "S" phaseolin type from Middle America into the Andes. However, phaseolin patterns and seed size did not reveal at what time and along which route genotypes were exchanged between Middle America and the Andes. This study demonstrates the usefulness of electrophoretic techniques applied to seed proteins for the study of domestication of the common bean. It emphasizes the importance of wild beans and landraces and stresses the need for additional germplasm collections specially of wild beans in the Andes.

The different geographical origin of the various phaseolin types, would permit to follow the world-wide dispersal of common bean cultivars from their centres of domestication, using phaseolin electrophoretic type on a marker. Both, Middle America and Andean cultivars were disseminated to different parts of the world. Middle American cultivars became the major component of the cultivar complement of two regions, Lowland South America and Southwest of the USA. The majority of the cultivars of Western Europe, the Iberian Peninsula, Africa and Northeastern USA originated in the Andes. The discovery of the Americas originated a rapid exchange of crops between the Old and the New World. In particular, common bean was first described in Europe around 1540. Evans (1976) suggested that the common bean had been introduced into Africa from Brazil during the slave trade. Portuguese traders might have introduced common bean cultivars from the Iberian Peninsula.

The sample of the Iberian Peninsula included a large proportion of "C" phaseolin cultivars. In the Americas, a comparable high frequency was found only in Chile. Chilean genotypes may have had a competitive advantage over genotypes of other origins because of a more adequate photoperiodic adaptation due to similar latitudes. Conversely, emigrants may have introduced Iberian cultivars into Chile. The "T" phaseolin type predominated among western European cultivars, genotypes originating in the Andean mountains may have been better adapted to cool summers of western Europe than their Middle America counterparts. Alternatively, the high frequency of "T" phaseolin cultivars is due to the high proportion of cultivars grown for their green pods.

The genetic diversity for a single trait —phaseolin seed storage protein— is very informative with respect to the domestication and dissemination patterns of common bean cultivars and complements archaeological, historical and linguistic data. A

first characteristic is obviously its polymorphism. A high level of variability has been observed among wild common beans. The second characteristic is its environmental stability. Perhaps the most important characteristic is the complexity of phaseolin at the molecular level. It is this complexity at the molecular level that allows phaseolin to be a very useful trait for detecting evolutionary patterns.

A more complete screening of phaseolin variability is needed, especially from areas insufficiently represented. The phaseolin gene family may be a good model to study the molecular evolution in plants. Phaseolin variability could be correlated with the traits at the molecular level, such as isozymes and DNA sequence.

PREPARATION OF FLOUR SAMPLES FOR ELECTROPHORESIS

Five seeds of each accession were analysed by 1 dimensional SDS/PAGE. A cotyledon sample, without embryo and tegument, had been grinded in a mortar until to get a flour. Initially, 0.1 - 0.2 g from the flour sample of each seed was suspended for at least 0.5 h in a 0.5 M NaCl solution (pH 2.4) and an equal volume of cracking buffer (0.625 M Tris Hcl pH 6.8; 2mM EDTA; 2 % SDS; 40 % Sucrose; 1 % 2-mercaptoethanol and 0.01 % bromophenol marker dye) (Brown *et al.*, 1981b) at room temperature under constant shaking. The suspension was centrifuged at 12000 rpm for 15 min and the supernatant was heat-treated at 100 ºC for 5 min, centrifuged at 13000 rpm for 15 min and submitted to electrophoresis.

ELECTROPHORESIS

1D-SDS-PAGE was performed according to the method for Laemmli (1970) modified by Ma and Bliss (1978). Electrophoresis was carried out in 0.75 mm thick, 15 % (w/v) polyacrilamide slab gels (Table 8) (Figure 11). The electrophoresis has been carried out at 25 mA for 4 h (Figure 12) until samples had gone thoughed stacking gel and at 30 mA for 18 h, during this time samples were migrated though separation gel. The gel had 1mm de grosor and the banding patterns have been observed by soaking in a solution of Coomassie 0,1 % (0,1 % Brillant Blue R, 40 % methanol, 10 % acetic acid), and have been fixed in a mixed of methanol (40 %) and acetic acid (10 %).

Table 7. Geographical distribution of phaseolin types in wild and cultivated common beans from Middle America and the Andes (Gepts *et al.*, 1986).

Region	Wild	Cultivated
Middle America	"S", "M"	"S" (92%), "T" (8%)
Colombia	"B", "CH"	"S" (64%), "T" (26%), "C" (7%), "B" (3%)
Andes	"T"	"T" (50%), "C" (23%), "H" (8%), "A" (2%)

Table 8. Runing and stacking gel recipes for 1 mm thick gels.

Reagents (4 gels)	4% Stacking Gel	15% Separation Gel
Distiled water	24 ml	23 ml
0,5M Tris HCl pH 6,8	10 ml	———
3,5M Tris HCl pH 8,8	———	25 ml
10% SDS	400 ul	1000 l
30% Acrylamide / Bis	5,2 ml	50 ml
10% Ammonium Persulfate	200 l	500 l
99% TEMED	40 l	50 l

Figure 11. Phaseolin patterns in polyacrilamide gels

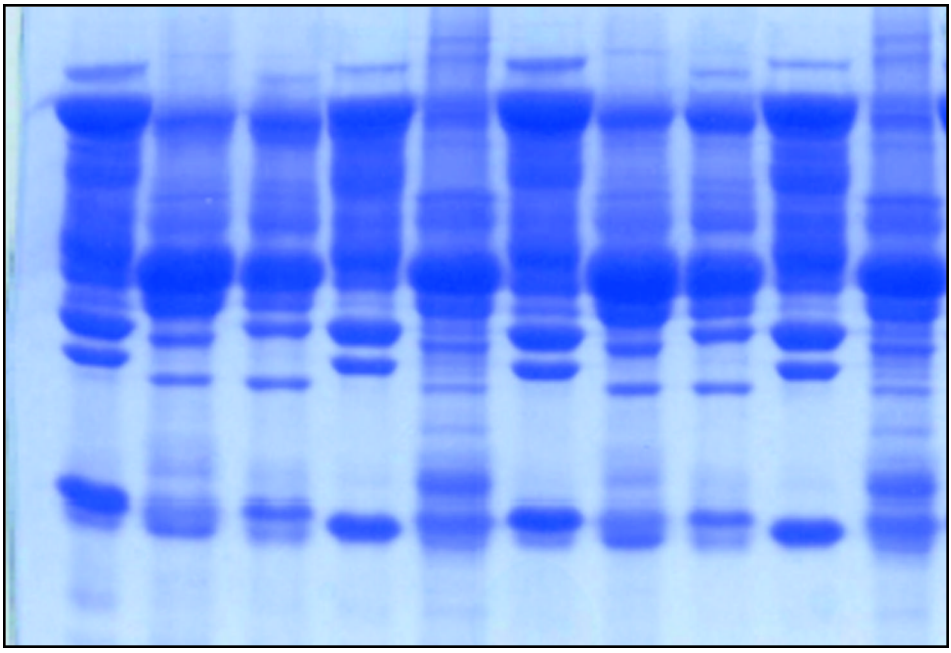

Figure 12. Vertical slab gel unit